Rec'd PCT/PTO 05 JUN 2001

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P66710US0
		US APPLICATION NO (If known, see 37 CFR 1 5) 09/856182
INTERNATIONAL APPLICATION NO PCT/GB99/04076	INTERNATIONAL FILING DATE 6 December 1999	PRIORITY DATE CLAIMED 5 December 1998
TITLE OF INVENTION MICROCELLULAR POLYMERS AS CELL GROWTH MEDIA AND NOVEL POLYMERS		
APPLICANT(S) FOR DO/EO/US Galip AKAY, Sandra DOWNES and Victoria Jane PRICE		

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    PCT/IB/306 Form
    First Page of Publication
    Amended Claims of the International Examination Preliminary Report

US APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/856182	PCT/GB99/04076	P66710US0

	CALCULATIONS	PTO USE ONLY
17. ■ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) .. $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .. $710.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$1000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$860.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1000.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ■ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**25** - 20 =	-5-	x $18.00	$ 90.00	
Independent Claims	**2** - 3 =	-0-	x $80.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1220.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 1220.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 1220.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 1220.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ■ A check in the amount of $ 1220.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

JACOBSON HOLMAN PLLC
400 7th Street, N.W., Suite 600
Washington, DC 20004
202-638-6666
CUSTOMER NUMBER: 00136

By Jonathan L Scherer Reg No 29,851 for
Michael R. Slobasky
Reg. No. 26,421

JPH&S 3/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Galip AKAY et al.

Serial No.: New

Filing Date: June 5, 2001

For: MICROCELLULAR POLYMERS AS CELL GROWTH MEDIA AND NOVEL POLYMERS

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend claims 3 through 7, 9 through 15, 18 through 21, 23 and 25 as follows:

3. (amended) Microcellular polyhipe polymer scaffold according to Claim 1 suitable for growth of living matter selected from cells, micro-organisms such as bacteria and virus and mixtures thereof.

4. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 comprising micro channels formed of pores with interconnects suitable for providing communication and penetration of living matter for anisotropic (directional) growth thereof.

5. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 wherein the walls of the micro-channels are (bio)degradable

suitable for fusion of living matter in the (bio)degraded scaffold.

6. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 comprising in individual zones, pore and interconnect sizes in different ranges, suitable for co-culturing two or more types of living matter.

7. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 wherein ratio of interconnect to pore diameter is in the range $0 < d/D < 0.5$, preferably in the range $0.1 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron

9. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 7 wherein the interface between a microcapillary wall and the bulk polymer provides a thin surface layer of the order of 0.5-5 micron, forming a zone particularly suited for directional (anisotropic) growth of living matter.

10. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 9 wherein the interface has smaller pore size than the bulk polymer wherein the zone is suitable for growth of cells forming a lining, for example cells lining the blood vessels or for growing endothelial cells on the interface surface.

11. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 comprising a module of shell and tube type or cubic/polyhedric type with respect to direction and/or configuration of channels and/or microcapillaries.

12. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 comprising a surface coating, using coating materials

introduced in situ during polymerisation or post polymerisation

13. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 wherein polymer is selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

14. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 wherein polymer comprises resiliently deformable or elastic material or is rendered resiliently deformable or elastic and is suitable for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth facilitating rate of cell growth.

15. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 wherein polyhipe scaffold is electrically conductive or is rendered electrically conductive whereby it is suitable for conducting an electric current during cell growth, facilitating distinguishing certain cell types and promoting growth and fusion of particular cell types

18. (amended) Process as claimed in Claim 16 comprising co-extrusion of polyhipe emulsions of differing pore and interconnect sizes eg concentrically or side-by-side.

19. (amended) Process as claimed in Claim 16 wherein the emulsion comprises aqueous and non-aqueous phases, preferably aqueous and oil.

20. (amended) A biologically active system comprising a polyhipe scaffold as defined in Claim 1 and living matter providing normal

cell functioning associated with a natural biologically active system present in the human or animal body, wherein living matter is selected from microorganisms or multiple cells selected from human, animal and plant cells, preferably selected from isotropic tissue and bone cells present in cartillage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells, of cell type selected from fibroblasts, chrondocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes neurons, myoblasts, macrophages and microvascular endothelium cells.

21. (amended) Method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined in Claim 1 comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth and providing conditions for growth promotion and positional control.

23. (amended) An organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in a polyhipe scaffold as defined in Claim 3 suited for growth of specific organ cells in the polyhipe and/or the channels, wherein cells are optionally in contact with a specific microchannel and all cells are capable of intercell communication.

25. (amended) The use of a polyhipe scaffold, a biologically active system, or organ support module as defined in Claim 1 for the manufacture of contact lenses, dental fillings, cochlea implants, vascular supports including heart valves and cardiac pace makers and drug delivery skin patches.

REMARKS

The foregoing Preliminary Amendment is requested in order to delete the multiple dependent claims and avoid paying the multiple dependent claims fee.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Early action on the merits is respectfully requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By Jonathan J. Skaer Reg No 29881 for
Michael R. Slobasky
Reg. No. 26,421

400 Seventh Street, N.W.
Washington, D.C. 20004-2201
(202) 638-6666

Atty. Docket: P66710US0
Date: June 5, 2001
MRS/cmf

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

3. (amended) Microcellular polyhipe polymer scaffold according to Claim 1 [or 2] suitable for growth of living matter selected from cells, micro-organisms such as bacteria and virus and mixtures thereof.

4. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 3] comprising micro channels formed of pores with interconnects suitable for providing communication and penetration of living matter for anisotropic (directional) growth thereof.

5. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 4] wherein the walls of the micro-channels are (bio)degradable suitable for fusion of living matter in the (bio)degraded scaffold.

6. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 5] comprising in individual zones, pore and interconnect sizes in different ranges, suitable for co-culturing two or more types of living matter.

7. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 6] wherein ratio of interconnect to pore diameter is in the range $0 < d/D < 0.5$, preferably in the range $0.1 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron

9. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 7 [any of Claims 7 and 8] wherein the interface between a microcapillary wall and the bulk polymer provides a thin surface layer of the order of 0.5-5 micron, forming a zone particularly suited for directional (anisotropic) growth of living matter.

10. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 9 [as dependent on Claim 3] wherein the interface has smaller pore size than the bulk polymer wherein the zone is suitable for growth of cells forming a lining, for example cells lining the blood vessels or for growing endothelial cells on the interface surface.

11. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 10] comprising a module of shell and tube type or cubic/polyhedric type with respect to direction and/or configuration of channels and/or microcapillaries.

12. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 11] comprising a surface coating, using coating materials introduced in situ during polymerisation or post polymerisation

13. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 12] wherein polymer is selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

14. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 13] wherein polymer comprises

resiliently deformable or elastic material or is rendered resiliently deformable or elastic and is suitable for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth facilitating rate of cell growth.

15. (amended) Microcellular polyhipe polymer scaffold as claimed in Claim 1 [any of Claims 1 to 14] wherein polyhipe scaffold is electrically conductive or is rendered electrically conductive whereby it is suitable for conducting an electric current during cell growth, facilitating distinguishing certain cell types and promoting growth and fusion of particular cell types

18. (amended) Process as claimed in Claim 16 [Claims 16 or 17] comprising co-extrusion of polyhipe emulsions of differing pore and interconnect sizes eg concentrically or side-by-side.

19. (amended) Process as claimed in Claim 16 [Claims 16 to 18] wherein the emulsion comprises aqueous and non-aqueous phases, preferably aqueous and oil.

20. (amended) A biologically active system comprising a polyhipe scaffold as defined in Claim 1 [any of Claims 1 to 15] and living matter providing normal cell functioning associated with a natural biologically active system present in the human or animal body, wherein living matter is selected from microorganisms or multiple cells selected from human, animal and plant cells, preferably selected from isotropic tissue and bone cells present in cartillage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells, of cell type selected from fibroblasts, chrondocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes neurons, myoblasts, macrophages and microvascular endothelium cells.

21. (amended) Method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined in Claim_1 [any of Claims 1 to 15] comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth and providing conditions for growth promotion and positional control.

23. (amended) An organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in a polyhipe scaffold as defined in Claim_3 [any of Claims 3 to 15] suited for growth of specific organ cells in the polyhipe and/or the channels, wherein cells are optionally in contact with a specific microchannel and all cells are capable of intercell communication.

25. (amended) The use of a polyhipe scaffold, a biologically active system, or organ support module as defined in Claim_1 [any of Claims 1 to 15, 20 and 23] for the manufacture of contact lenses, dental fillings, cochlea implants, vascular supports including heart valves and cardiac pace makers and drug delivery skin patches.

PATENT
ATTY. DOCKET NO.: P66710US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Galip AKAY et al.

Serial No.: 09/856,182

Filed: June 5, 2001

For: MICROCELLULAR POLYMERS AS CELL GROWTH MEDIA AND NOVEL POLYMERS

SECOND PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, kindly amend the above-identified application as follows:

IN THE SPECIFICATION:

Please amend the specification at page 1, after the title, insert the following new paragraph:

--This application is the national phase under 35 USC §371 of PCT International application No. PCT/GB99/04076 which has an International Filing Date of December 6, 1999, which designated the United States of America and was published in English and claims priority from 9826701.6 filed December 5, 1998 in the United Kingdom.--

IN THE CLAIMS:

Please cancel claims 1-25 without prejudice or disclaimer.

Please insert new claims 26-61 as follows:

--26. Micro cellular polyhipe polymer scaffold suitable for growth of living matter for biomedical applications, comprising a homogeneous cross linked open cellular material defined by a bulk polymer matrix having a surface and an interface with an internal phase, and having porosity greater than 75% comprising emulsion derived pores of diameter in the range of 0.1 to 10,000 micron and emulsion derived pore interconnects of diameter in the range of up to 100 micron, wherein the scaffold comprises a plurality of discrete zones with location selected from:

at the polymer surface;

within its bulk matrix;

at the interface between polymer and internal phase; and

between adjacent but distinct pores or interconnects, characterized by form and dimension of pore and interconnect type within each zone, and location of zones wherein adjacent zones are distinguished by boundaries, whereby zones are suitable for regulating positioning and morphology of living matter, wherein the scaffold comprises controlled pore sizes selected from the range up to 0.5 μm, up to 300 μm, up to 10,000 μm, and up to nm size and comprises pore interconnects selected from the range up to 100 micron, and approaching 500 micron, characterized in that the scaffold comprises pore and interconnect sizes in different ranges in two or more distinct zones.

27. Microcellular polyhipe polymer scaffold as claimed in Claim 26 wherein discrete zones are interpenetrating.

28. Microcellular polyhipe polymer scaffold as claimed in Claim 27 obtainable by polymerizing a high internal phase emulsion of an immiscible dispersed phase in a continuous phase, wherein the dispersed phase is void or contains dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled emulsification temperature and rate to achieve an emulsion of controlled pore size, and subsequently homogenizing for controlled period under controlled deformation and polymerising under controlled temperature and pressure, characterized in that controlled pore size approaching 0.1 μm up to 0.5 μm is obtained using very high deformation rate flows in which the flow is predominantly extensional and low emulsification temperature, pore size up to 300 μm is obtained using rate just above the critical deformation rate at which phase inversion takes place and high emulsification temperature, very large pore size up to 10,000 μm is obtained through the method of controlled pore coalescence during polymerization, and nano-pore size up to nm is obtained through solvent extraction after polymerization; and in that microcapillaries of diameter in the range 10 – 1000 μm is obtained by polymerising about a 3D network of fibers and in that differing pore and interconnect sizes are obtained by co-extrusion of polyhipe emulsions.

29. Microcellular polyhipe polymer scaffold as claimed in Claim 28 wherein microcapillary networks are present within the emulsion derived pores.

30. Micro cellular polyhipe polymer scaffold as claimed in Claim 29 comprising more than one type of microcapillary.

31. Micro cellular polyhipe polymer scaffold as claimed in Claim 30 wherein each microcapillary type is distinguished by diameter, surface modification, interface porosity or pore size or chemical structure.

32. Micro cellular polyhipe polymer scaffold as claimed in Claim 31 wherein emulsion derived pores comprise nano-porous walls which are void, increasing the size of interconnects or which contain filler polymers for extra strength.

33. Microcellular polyhipe polymer scaffold as claimed in Claim 32 suitable for growth of living matter selected from cells, micro-organisms such as bacteria and virus and mixtures thereof.

34. Microcellular polyhipe polymer scaffold as claimed in Claim 33 comprising micro channels formed of pores with interconnects suitable for providing communication and penetration of living matter for anisotropic (directional) growth thereof.

35. Microcellular polyhipe polymer scaffold as claimed in Claim 34 wherein the walls of the micro-channels are (bio)degradable suitable for fusion of living matter in the (bio)degraded scaffold.

36. Microcellular polyhipe polymer scaffold as claimed in Claim 35 comprising in individual zones, pore and interconnect sizes in different ranges, suitable for co-culturing two or more types of living matter.

37. Microcellular polyhipe polymer scaffold as claimed in Claim 36 wherein ratio of interconnect to pore diameter is in the range $0 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron.

38. Microcellular polyhipe polymer scaffold as claimed in Claim 37 comprising extensive networks of elongate micro capillaries obtainable by moulding about fibrous inserts of diameter in the range from 10 micron up to 1000 micron, throughout the scaffold or zones thereof, separated by the microcellular polymer wherein microcapillaries are suitable for blood or nutrient supply channels, expression channels for living matter and seeding of living matter.

39. Microcellular polyhipe polymer scaffold as claimed in Claim 38 wherein the interface between a microcapillary wall and the bulk polymer provides a thin surface layer of the order of 0.5-5 micron, forming a zone particularly suited for directional (anisotropic) growth of living matter.

40. Microcellular polyhipe polymer scaffold as claimed in Claim 39 wherein the interface has smaller pore size than the bulk polymer wherein the zone is suitable for growth of cells forming a

lining, for example cells lining the blood vessels or for growing endothelial cells on the interface surface.

41. Microcellular polyhipe polymer scaffold as claimed in Claim 40 comprising a module of shell and tube type or cubic/polyhedric type with respect to direction and/or configuration of channels and/or microcapillaries.

42. Microcellular polyhipe polymer scaffold as claimed in Claim 41 comprising a surface coating, using coating materials introduced in situ during polymerization or post polymerization

43. Microcellular polyhipe polymer scaffold as claimed in Claim 42 wherein polymer is selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

44. Microcellular polyhipe polymer scaffold as claimed in Claim 43 wherein polymer comprises resiliently deformable or elastic material or is rendered resiliently deformable or elastic and is suitable for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth facilitating rate of cell growth.

45. Microcellular polyhipe polymer scaffold as claimed in Claim 44 wherein polyhipe scaffold is electrically conductive or is rendered electrically conductive whereby it is suitable for conducting an electric current during cell growth, facilitating distinguishing certain cell types and promoting growth and fusion of particular cell types

46. A process for the preparation of a microcellular polyhipe polymer scaffold as hereinbefore defined comprising in a first stage the formation of a high internal phase emulsion (HIPE) of an immiscible dispersed phase in a continuous phase, wherein the dispersed phase is void or contains dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature and rate to achieve an emulsion of controlled pore size, and subsequently homogenising for controlled period under controlled deformation and polymerising under controlled temperature and pressure wherein controlled pore size of emulsions up to 0.5 μm are obtained using very high deformation rate flows in which the flow is substantially extensional and high emulsification temperature, pore size up to 300 μm are obtained using rate just above the critical deformation rate at which phase inversion takes place, very large pore size up to 10,000 μm are obtained through the method of controlled pore coalescence during polymerisation, and nano-pore size up to nm are obtained through solvent extraction after polymerization and microcapillaries are obtained by polymerising about a 3D network of fibers.

47. Process as claimed in Claim 46 wherein very large pore size up to 10,000 μm are obtained by adding water soluble polymer to the aqueous phase or filler solutes to the oil phase at elevated concentrations with controlled pore coalescence during polymerization.

48. Process as claimed in Claim 47 wherein nano-pore size up to nm are obtained using an oil phase filler selected from high boiling point hydrocarbon, another monomer or macromonomer, reactive or inert polymer and/or solid particles optionally with solvent extraction after polymerization.

49. Process as claimed in Claim 48 comprising co-extrusion of polyhipe emulsions of differing pore and interconnect sizes eg concentrically or side-by-side.

50. Process as claimed in Claim 49 using multiple-feed points with a prolonged dosing to create a large pore emulsion.

51. Process as claimed in Claim 50 wherein emulsification temperature is greater than 60C.

52. Process as claimed in Claim 51 wherein homogenisation temperature is in the range 60 - 150C.

53. Process as claimed in Claim 52 carried out with use of additional oil phase initiator.

54. Process as claimed in Claim 53 carried out with use of additional oil phase filler.

55. Process as claimed in Claim 54 wherein the emulsion comprises aqueous and non-aqueous phases.

56. A biologically active system comprising a polyhipe scaffold as defined in Claim 26 and living matter providing normal cell functioning associated with a natural biologically active system present in the human or animal body, wherein living matter is selected from microorganisms or multiple cells selected from human, animal and plant cells, preferably selected from isotropic tissue and bone cells present in cartillage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells, of cell type selected from fibroblasts, chrondocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes neurons, myoblasts, macrophages and microvascular endothelium cells.

57. Method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined in Claim 26 comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth and providing conditions for growth promotion and positional control.

58. Use of a biologically active system as defined in Claim 56 as an implant or in association in vivo in or with the human or animal body or as a module for in vitro studies mimicking a part of the human or animal body or for use in a growth environment, for example for the growth of organ cells in the cell side of the module in order to stimulate organs.

59. An organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in a polyhipe scaffold as defined in Claim 26 suited for growth of specific organ cells in the polyhipe and/or the channels, wherein cells are optionally in contact with a specific microchannel and all cells are capable of intercell communication.

60. A method for manufacturing an organ support module as defined in Claim 59 comprising providing a polyhipe emulsion, providing a mould including inserts such as rods or fine fibres, pumping polyhipe emulsion and optional filler into the mould about the inserts and polymerising, with subsequent removal of inserts and optional filler to provide pores and interconnects, microcapillaries and nano-pores in desired configuration.

61. The use of a polyhipe scaffold, a biologically active system, or organ support module as defined in Claim 26, for the manufacture of contact lenses, dental fillings, cochlea implants, vascular supports including heart valves and cardiac pace makers and drug delivery skin patches.

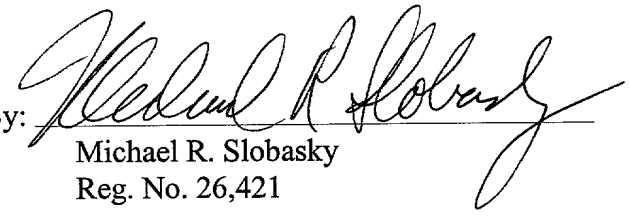
REMARKS

In view of the above amendments, favorable consideration in this application is respectfully requested. By this Amendment, claims 1-25 have been canceled without prejudice or disclaimer and new claims 26-61 have been added. Claims 26-61 remain in the application, with claims 26 and 46 being independent.

Based upon the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for examination. Should the Examiner have any questions or comments, the Examiner is cordially invited to telephone the undersigned attorney.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By: 

Michael R. Slobasky
Reg. No. 26,421

400 Seventh Street, N. W.
Washington, D.C. 20004
Telephone: (202) 638-6666
Atty. Docket No.: P66710US0
Date: September 5, 2001
MRS:cwp
A:\P66710 PAM

45 46

CLAIMS

1. Micro cellular polyhipe polymer scaffold suitable for growth of living matter for biomedical applications,
comprising a homogeneous cross linked open cellular material defined by a bulk polymer matrix having a surface and an interface with an internal phase, and having porosity greater than 75% comprising emulsion derived pores of diameter in the range of 0.1 to 10,000 micron and emulsion derived pore interconnects of diameter in the range of up to 100 micron, wherein the scaffold comprises a plurality of discrete and/or interpenetrating zones:
at the polymer surface;
within its bulk matrix;
at the interface between polymer and internal phase; and/or
between adjacent but distinct pores and/or interconnects,
characterised by form and dimension of pore and interconnect type within each zone, and location of zones wherein adjacent or interpenetrating zones are distinguished by boundaries which may be between or contained within adjacent pores and/or interconnects in respective zones, whereby zones are suitable for regulating positioning and/or morphology of living matter
comprising controlled pore sizes in the range up to 0.5 μm, up to 300 μm, up to 10,000 μm, and/or up to nm size and comprising pore interconnects in the range up to 100 micron, and/or approaching 500 micron
with the proviso that in the case that pore type is present having size in the range 1 – 50 micron, at least one additional pore type is present in a range as herein defined other than 1 – 50 micron.

2. Microcellular polyhipe polymer scaffold according to Claim 1 obtainable by polymerising a high internal phase emulsion of an immiscible dispersed phase in a continuous phase, wherein the dispersed phase is void or contains

46 47

dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature and rate to achieve an emulsion of controlled pore size, and subsequently homogenising for controlled period under controlled deformation and polymerising under controlled temperature and pressure,

characterised in that controlled pore size up to 0.5 μm is obtained using very high deformation rate flows in which the flow is predominantly extensional and low emulsification temperature, pore size up to 300 μm is obtained using rate just above the critical deformation rate at which phase inversion takes place and high emulsification temperature, very large pore size up to 10,000 μm is obtained through the method of controlled pore coalescence during polymerisation, and nano-pore size up to nm is obtained through solvent extraction after polymerisation; and in that controlled interconnect diameter up to 500 micron is obtained by polymerising about a 3D network of fibres.

3. Microcellular polyhipe polymer scaffold according to Claim 1 *or 2* suitable for growth of living matter selected from cells, micro-organisms such as bacteria and virus and mixtures thereof.

4. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 3 comprising micro channels formed of pores with interconnects suitable for providing communication and penetration of living matter for anisotropic (directional) growth thereof.

5. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 4 wherein the walls of the micro-channels are (bio)degradable suitable for fusion of living matter in the (bio)degraded scaffold.

47 48

6. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 5 comprising in individual zones, pore and interconnect sizes in different ranges, suitable for co-culturing two or more types of living matter.

7. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 6 wherein ratio of interconnect to pore diameter is in the range $0 < d/D < 0.5$, preferably in the range $0.1 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron

8. Microcellular polyhipe polymer scaffold as claimed in Claim 7 comprising extensive networks of elongate micro capillaries obtainable by moulding about fibrous inserts of diameter as low as 10 micron, throughout the scaffold or zones thereof, separated by the microcellular polymer wherein microcapillaries are suitable for blood or nutrient supply channels, expression channels for living matter and seeding of living matter.

9. Microcellular polyhipe polymer scaffold as claimed in any of Claims 7 and 8 wherein the interface between a microcapillary wall and the bulk polymer provides a thin surface layer of the order of 0.5-5 micron, forming a zone particularly suited for directional (anisotropic) growth of living matter.

10. Microcellular polyhipe polymer scaffold as claimed in Claim 9 as dependent on Claim 3 wherein the interface has smaller pore size than the bulk polymer wherein the zone is suitable for growth of cells forming a lining, for example cells lining the blood vessels or for growing endothelial cells on the interface surface.

11. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 10 comprising a module of shell and tube type or cubic/polyhedric type with respect to direction and/or configuration of channels and/or microcapillaries.

~~48~~ 49

12. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 11 comprising a surface coating, using coating materials introduced *in situ* during polymerisation or post polymerisation

13. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 12 wherein polymer is selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

14. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 13 wherein polymer comprises resiliently deformable or elastic material or is rendered resiliently deformable or elastic and is suitable for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth facilitating rate of cell growth.

15. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 14 wherein polyhipe scaffold is electrically conductive or is rendered electrically conductive whereby it is suitable for conducting an electric current during cell growth, facilitating distinguishing certain cell types and promoting growth and fusion of particular cell types

16. A process for the preparation of a microcellular polyhipe polymer scaffold as hereinbefore defined comprising in a first stage the formation of a high internal phase emulsion (HIPE) of an immiscible dispersed phase in a continuous phase, wherein the dispersed phase is void or contains dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature and rate to achieve an emulsion of controlled pore size,

49 50

and subsequently homogenising for controlled period under controlled deformation and polymerising under controlled temperature and pressure.

17. Process as claim in Claim 16 wherein controlled pore size of emulsions up to 0.5 μm are obtained using very high deformation rate flows, pore size up to 300 μm are obtained using rate just above the critical deformation rate at which phase inversion takes place, very large pore size up to 10,000 μm are obtained through the method of controlled pore coalescence during polymerisation, and nano-pore size up to nm are obtained through solvent extraction after polymerisation.

18. Process as claimed in Claims 16 or 17 comprising co-extrusion of polyhipe emulsions of differing pore and interconnect sizes eg concentrically or side-by-side.

19. Process as claimed in Claims 16 to 18 wherein the emulsion comprises aqueous and non-aqueous phases, preferably aqueous and oil.

20. A biologically active system comprising a polyhipe scaffold as defined in any of Claims 1 to 15 and living matter providing normal cell functioning associated with a natural biologically active system present in the human or animal body, wherein living matter is selected from microorganisms or multiple cells selected from human, animal and plant cells, preferably selected from isotropic tissue and bone cells present in cartillage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells, of cell type selected from fibroblasts, chrondocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes neurons, myoblasts, macrophages and microvascular endothelium cells.

50 51

21. Method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined in any of Claims 1 to 15 comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth and providing conditions for growth promotion and positional control.

22. Use of a biologically active system as defined in Claim 20 as an implant or in association *in vivo* in or with the human or animal body or as a module for *in vitro* studies mimicking a part of the human or animal body or for use in a growth environment, for example for the growth of organ cells in the cell side of the module in order to stimulate organs.

23. An organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in a polyhipe scaffold as defined in any of Claims 3 to 15 suited for growth of specific organ cells in the polyhipe and/or the channels, wherein cells are optionally in contact with a specific microchannel and all cells are capable of intercell communication.

24. A method for manufacturing an organ support module as defined in Claim 23 comprising providing a polyhipe emulsion, providing a mould including inserts such as rods or fine fibres, pumping polyhipe emulsion and optional filler into the mould about the inserts and polymerising, with subsequent removal of inserts and optional filler to provide pores and interconnects, microcapillaries and nano pores in desired configuration.

25. The use of a polyhipe scaffold, a biologically active system, or organ support module as defined in any of Claims 1 to 15, 20 and 23 for the manufacture of contact lenses, dental fillings, cochlea implants, vascular

supports including heart valves and cardiac pace makers and drug delivery skin patches.

MICROCELLULAR POLYMERS AS CELL GROWTH MEDIA AND NOVEL POLYMERS

The present invention relates to microcellular polymers as cell growth media, biologically active systems and organ support modules comprising the polymers and biological cells, a method for cell growth in the materials, the use thereof as implants in the human or animal body or for *in vitro* studies, novel processes for the preparation and modification of microcellular polymers and the polymers obtained thereby and a method for manufacture of an organ support module.

More specifically the present invention relates to microcellular polymers having porosity in excess of 75%, in the form of a pore and pore interconnect structure having relative interconnect and pore dimensions d/D as cell growth media for three dimensional cell growth throughout the polymer, the system comprising this, method for growth and use thereof, and novel processes for the preparation thereof and polymers obtained thereby.

Microcellular synthetic organic/inorganic/natural materials in which the pores are interconnected are often used as a support to grow plant or animal cells or enzymes.

Tailoring of a microcellular support for a specific cell growth application is often difficult with naturally forming microcellular materials. Recently efforts have been made to grow cells on newly developed microcellular materials, which are prepared through a high internal phase emulsion (HIPE) polymerisation route in which the phase volume of the dispersed phase is

greater than about 75% and subsequently polymerising and/or cross linking to obtain a rigid structure, offering many advantages. These materials have been described both in open literature and in patent disclosures, and they are referred to as polyhipe polymers (PHPs).

It is stated that the important features of these materials are:

1. The pore volume can be as high as 97% (for practical purposes);
2. Pores are interconnected;
3. Pore and interconnect sizes can be controlled accurately and independently;
4. Additional polycondensation reactions can be achieved;
5. Crosslinking can be achieved with proteins, polymers, silicates or organic polymers plus a wide range of porous materials can be obtained;
6. Post polymerisation/crosslinking modification of PHP is possible which is further facilitated by using functional monomers at the emulsification stage;
7. PHP can be obtained in block and/or in particulate form;
8. Since HIPEs can be pumped, it is possible to form moulded structures.

US patent 5,071,747 describes the preparation of microcellular polymeric support materials with average void (pore) diameter within a range of from 1 to 150 μm interconnected by holes (interconnects). Hole size (d) is related to the void size (D) and their ratio can be controlled in the range of $0 < d/D < 0.3$. The control of d/D ratio is through the control of the surfactant concentration and by the addition of electrolyte, mainly $CaCl_2$ in the range

of 10^{-4} molar to 5 molar. The electrolytes are selected from soluble halides and sulphates. The function of the electrolyte is claimed to be the control of the size of the holes and to improve stability of the emulsion.

In stage-1, the oil and aqueous phases are introduced under deformation (agitation) and in stage-2, the resulting HIPE is homogenised under deformation (agitation). Although the rate of introduction of the phases (i.e. dosing time (t_D)) of the dispersed phase into the batch mixer, and the subsequent homogenisation time (t_H) of HIPE is quoted in US patent 5,071,747, the mixing conditions are not specified. Polymerisation is followed by introduction of cells.

US 5,071,747 discloses isotropic (non-directional) plant and animal cell growth in three dimensions (3D) in polyvinyl Polyhipe Polymer (PHP), the generic name for the micro porous material. Reactants or nutrients are provided via inter connecting holes (interconnects) within the PHP to access voids (pores) in which cells are grown or reacted to produce products. The PHP is used purely as a cell growth medium allowing normal cell functioning within voids, using holes (interconnects) as access channels. Voids and holes were provided having void diameter of 6-12 times the diameter of cells to be introduced and grown and hole diameter of 3-6 times, i.e. void diameter 45 micron and hole diameter 15 micron in the case of growth of yeast cells of the order 5 micron. In these cases, however cell growth was predominantly surface growth. In a further example PHP having porosity of 90%, void and hole diameter of 30 micron and 10 micron respectively was used for fungal growth which was found to penetrate the porous polymer network.

Nevertheless the literature gives only limited teaching with regard to controlling pore and interconnect size. In US 5,071,747 above it is not stated how the claimed dimensions were achieved and in fact it would not be possible to obtain pore dimensions in excess of 50 microns using the limited information given, nor would it be possible to control or pre-determine a particular pore size within the range 1-50 microns without extensive experimentation.

The usefulness of PHP materials for cell growth remains therefore extremely limited and there is a need for materials and methods for preparing more sophisticated polymers enabling more sophisticated cell growth.

We have now surprisingly found that coherent cell growth may be achieved within PHP polymers to provide a co-operating multi cell system which is adapted for a number of uses and that novel PHPs may be obtained having novel properties adapted for biological and non-biological uses. It is particularly advantageous that cells co-operate in the PHP of the invention as this is important for cell growth.

Accordingly in the broadest aspect of the invention there is provided micro cellular polyhipe natural or synthetic polymer in the form of a homogeneous cross linked open cellular material having porosity greater than 75%, comprising pores of diameter 1 to 10,000 micron and pore interconnects of diameter of up to 100 micron, wherein the polyhipe is a scaffold for multicell growth in three dimensions wherein the polymer pores and interconnects are comprised in a plurality of distinct or interpenetrating zones which are

adapted to regulate cell positioning and morphology whereby cell growth is constrained within and/or extends throughout plural zones in directional and/or nondirectional manner to provide a multiple cell structure for biomedical applications.

The polymer according to the invention may be provided with additional features in the form of micro capillary networks, nanopores, surface, chemical modification, electrical or like properties for specific purposes.

The invention thus provides useful PHP materials, the many applications of which are highly significant specifically the invention can be used as a multi-channel in-vitro organ module in which the cells must be under a certain chemical and/or electrical potential, to express and thus function. The chemical and/or electrical potential will be provided by the contents of the micro-channels. This type of module is necessary in organs like liver, kidney and pancreas. Modules with micro-channels can also be used as selective bio-reactors in which the cell expression is regulated through the presence of a chemical and/or electrical potential imposed through each type of channel content. Cell expression under different types of potential and potential strength can be used to produce biochemicals which can lead to the understanding of disease processes and/or preparation of new proteins in drug applications.

The use of PHP materials in the production of biochemicals is not confined to the animal cells grown on a support system with a preferred architecture and physiological conditions. Plant cells as well as micro-organisms such as bacteria and virus can also be grown using the PHP material. In this case

particulate form of the PHP material with open pores can be used to provide support for cell, bacteria or virus growth. The particulate PHP material can be suspended in a suitable biomedia for the biochemical reaction to proceed. Monolithic support systems such as those described in this invention can also be used for this purpose also. When viruses are to be grown on PHP support, small pore size material, less than 1 micron, may be preferred.

Preferably zones are adapted for growth of multiple cell types independently constrained within and/or extending throughout multiple zones respectively.

The important parameters that promote cell growth are the pore and interconnect sizes and chemical and physical characteristics of the support surface. In some cases, the cell growth may be anisotropic (directional) and therefore the pores of the cell support may be required to be in the form of micro-channels with interconnects to provide cell-communication and cell penetration. The walls of the micro-channels can be (bio)degradable so that subsequent cell fusion can be obtained after (bio)degradation.

Biomedical applications include any application in which the materials interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body. The materials may therefore be envisaged for a range of applications for example the manufacture of contact lenses, dental fillings, cochlea implants, vascular supports including heart valves and cardiac pace makers and drug delivery skin patches and the like.

Reference herein to a scaffold is to a porous material which provides support and allows a positioning or load bearing function on the scaffold at an early

stage as the scaffold directs growth or takes the load until cells are grown within a scaffold and have developed extracellular matrix to gain load bearing ability and with stand loads themselves.

Cell growth may be of cells which are introduced into the scaffold or migrate into the scaffold, for example in the case that a scaffold is used for synthetic growth or is used for natural cell growth by migration of cells from surrounding tissue.

Reference herein to polyhipe is to any natural or synthetic polymer comprising pores and interconnects as hereinbefore defined obtained by polymerising a high internal phase emulsion.

Reference herein to pores and interconnects in the polymer is to empty pores or cells with pore interconnects therebetween, which may be empty or may contain dissolved or dispersed materials. Pores and interconnects are distinguished by their relative magnitude and dimensions as herein below defined. The total porosity of the material is the combined empty space provided by the sum of all pores and interconnects.

Reference herein to zones within the polymer is to distinct or interpenetrating regions characterised by the form, location, magnitude or other property of pores and interconnects comprised in the zone. For example one or more zones are provided at the polymer surface, within its bulk matrix, at the interface between polymer and internal phase, between adjacent pores and/or interconnects of different form or dimension or adapted for growth of different cell types. Zones are distinguished by boundaries which may be

between or contained within adjacent pores and/or interconnects in respective zones. Cell support material with two or more distinct zones where the pore and interconnect sites are different will be needed when co-culturing two or more cell types.

Preferably polymer according to the invention is suited for growth of multiple cell types to provide a multi zone cell structure in which selected cell types are confined to the specific boundary whilst other cell types grow throughout the structure across boundaries presented by the scaffold. By this means the polyhipe scaffold provides for a multiple cell system extending throughout the scaffold, optionally including intrainterconnect or intramicrocapillary growth and exhibiting zoning and optionally interzoning of biological cells.

The polymer of the invention is moreover able to encourage natural zoning of cells by migration within, throughout and between zones which have been tailored for preferential migration of desired cell types.

The polymer of the present invention may be obtained from any desired natural or synthetic monomers, oligomers, macromonomers, reactive polymers and mixtures thereof which exhibit bio-compatability. Polyhipes are commercially available or may be prepared using methods as disclosed in US 5071747 and in additional patent publications referred therein or as hereinbelow described.

The generic polyhipe polymer which is commercially available comprises polyvinyl polyhipe and is made up of oil phase monomers styrene, divinyl

benzene (DVB) and surfactant (Span 80 sorbitan monooleate), and may be in rigid or flexible form depending on the relative proportions of monomers, additionally in flexible form including monomer 2-ethylhexyl acrylate, and in the aqueous phase an amount of potassium persulphate as aqueous phase initiator.

The polymer of the invention may be natural or synthetic, soluble or insoluble, optionally (bio)degradable crosslinked polymer, preferably selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

Commercially available polyhipe typically has pore diameter in the range 5-50 micron.

PHP support according to the invention may have pore diameter in the range 0.5 or 1-10,000 micron. A preferred lower range limit is 10 micron, more preferably 30, for example 50 micron. A preferred upper range is 300 micron, more preferably 200 micron. Polyhipe of particular pore diameter may be obtained by methods described hereinbelow, and may have any desired ratio of interconnect to pore diameter, for example in the range $0 < d/D < 0.5$, preferably in the range $0.1 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron. Interconnects may have diameter in a range of up to 100 micron, preferably 0.001 to 100 micron, more preferably 10-50 micron. Extensive networks of elongate micro capillaries of diameter as low as 10 micron may be provided, these capillaries being separated by the microcellular polymer. A zone comprising the interface between a

capillary wall and the bulk polymer may provide a surface layer of any desired thickness for growth of cells of any desired size. An interface having a thin surface layer of the order of 0.5-5 micron is particularly suited for growth of neurons (nerve cells) or muscle cells in which directionality is important. The interface having smaller pore size than the bulk provides an ideal zone for growth of cells forming a lining, for example cells lining the blood vessels or for growing endothelial cells on the interface surface.

Pore structure in the present invention are formed through 4 different mechanisms. These mechanisms are used, often in combination, to obtain several forms of support architecture in order to create a realistic model for organ support. Four different pore structures are described below:

Type - 1 Pores (Basic pores): This is the basic pore structure the size of which is determined at the emulsification stage of the PHP formation. Therefore, the pore size is mainly determined by the deformation (flow) history of the emulsion. The integrity of these pores are kept during polymerisation and the interconnects are formed at this stage. Depending on the chemistry of the oil and aqueous phases, phase volume and the polymerisation conditions such as temperature and pressure, the interconnect size can be controlled in the range $0<d/D<0.5$.

Type - 2 Pores (Coalescence pores): This type of pore architecture is obtained through the controlled coalescence of the Type-1 pores during polymerisation. The dispersed phase droplets in the emulsion of the PHP are coalesced by the addition of water soluble polymers into the aqueous phase, or by adding slightly hydrophilic oils (such as styrene oxide) to the oil

phase. The interconnect size in this case is the same as that of the Type-1 pores which form a matrix incorporating the coalesced pores. However, due to the fact that the coalesced pores are very large compared with the basic pores, the d/D ratio is very small.

Type 3 - Pores (Micro-capillaries): It is possible to obtain a network of microcapillaries within the Type-1 or Type-2 pores (i.e. supported by these pores) or indeed to obtain microcapillaries supported by Type-1 pores with Type-2 pores forming the walls of the capillaries or vice versa. These microcapillaries are formed using a specially constructed mould, in which rods or fine fibres are inserted. The PHP emulsion is pumped into the mould and polymerisation is carried out. After polymerisation, these inserts are removed either by pulling them out (when polymerisation results in shrinkage, or when the polymeric inserts are first swollen during polymerisation and subsequently shrunk upon polymerisation or the removal of the solvent used as filler for the monomer) or by dissolving the inserts in acid or a suitable solvent. The minimum diameter of these micro capillaries can be as low as 10 micron (using glass, carbon or polymeric fibres). Metallic fibres can be used to obtain microcapillaries in the range 50-1000 micron or greater.

Type - 4 Pores (Nano-pores): The pore walls of the microporous PHP can be made nano-porous by using a suitable 'filler' in the oil -phase so that, after polymerisation, the filler or its reacted form can be removed by solvent extraction. The filler can be oil or another monomer or macromonomer which do not undergo polymerisation or if the monomer/macromonomer polymerizes, the resultant polymer could be extracted. The filler can also be

another polymer or reactive polymer which dissolve in the monomer or a suitable hydrocarbon oil as well as nano-sized solids such as silica powder or zeolite. If the 'filler' oil is highly soluble in the monomer as well as the resulting crossed linked polymer, it will create very fine pores after extraction. If the resulting polymer is not soluble in the filler oil or if it does not swell, they will form sub-micron particles attached to each other by a number of crosslinking chains. This network of particles and the filler oil will form a co-continuous system. This method can be used to control the amount of nano-porosity in the micro-porous polymer. Nano-pores are also obtained by using polymers which are soluble in the monomer. These polymers can also be extracted after the formation of the polyhipe polymer to create nano-pores. Such filler polymers, if not extracted, can provide extra strength. Such nano-pores will allow the diffusion of small molecular weight solutes across the organ support since the walls will create a barrier for the transport of these vital solutes. Such solutes include oxygen, carbon dioxide, nutrients, growth hormones, electrolyte and the like. The presence of the nano-pores can also be useful in the controlled biodegradation of the organ support through hydrolytic polymer degradation since the effective surface area will be very high.

Pore types may be present in any modular form, for example shell and tube type or cubic/polyhedric.

Preferably pore size and inter connect size are selected according to the type of cell to be grown and the type of growth, i.e. with or without penetration, confined to a boundary or crossing boundaries between zones. Pore size in zones intended for growth of cells throughout the zone is preferably of

diameter 2 – 3 times the diameter of cells to be grown, for example 2.5 times cell diameter. Without being limited to this theory, it is thought that this ratio of dimensions is optimum for cell types, including cartilage cell types, whereby cells are able to grow and prosper, as indicated by production of collagen. For example cartilage cells of diameter 10 micron were grown in pores of diameter in the range 17-30 micron, specifically of 25 micron.

The polyhipe scaffold may be adapted to provide a desired surface characterised by means of the surface coating, using coating materials introduced *in situ* during polymerisation or post polymerisation. Any known materials typically used for coating polymers for use in cell growth may be employed, for example cell growth promoters such as hydroxylapatite or tricalcium phosphate, which also promotes biocompatability, other minerals, silica, collagen, hyaluran, polyethylene oxide, carboxymethyl cellulose (CMC), proteins, organic polymers, particles and the like. Coating *in situ* provides homogeneous coating throughout the scaffold. Post polymerisation coating, for example using laser ablation results in coating confined to the surface of the bulk matrix and may be particle coating.

The polyhipe scaffold may be constructed of any desired materials as hereinbefore defined. It has been found that cyclic mechanical straining of the support or the application of an electric field may also enhance the rate of cell growth. In most cases, the biodegradability of the support does not appear to be important and therefore the support material with growing cells can be implanted in the animal body without fear of rejection. However, when cell-cell fusion is required, (bio)degradability becomes important.

Therefore the polymer is preferably made of resiliently deformable or elastic materials or is rendered resiliently deformable or elastic by suitable means. Preferred materials are therefore thermoplastics which may be deformed in suitable manner to influence cell growth. Preferably a polyhipe support as hereinbefore defined is suited for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth. This has been found to have beneficial effects in cell growth rate promotion.

The polyhipe scaffold as hereinbefore defined may be electrically conductive or may be rendered electrically conductive by known means whereby it is adapted to conduct an electric current during cell growth. This technique is particularly advantageous for distinguishing certain cell types and promoting growth and fusion of particular cell types such as neurons and muscle cells.

The polyhipe scaffold may be biodegradable or may be rendered biodegradable by known means whereby it is adapted to be degraded by contact with any desired agent or by introduction into any desired environment, for example a specific biological agent or environment. Preferably the polyhipe scaffold is adapted for degradation by the hydrolysis or enzyme-catalysed hydrolysis of the polymer chains and cross link or by the erosion of the polymer. By this means the polyhipe scaffolds may be used as a scaffold to support a biologically active system for the duration of time required for that system to establish itself and be self supporting and thereafter degrade with no detrimental effects. Preferably a degradeable scaffold is used for growth of cell types including for example muscle cells. By this means a scaffold is adapted to position multiple cell types in pre determined zones and thereafter to degrade allowing self fusion to take place

in the absence of the intervening scaffold whereby functioning biologicially active micro systems are created.

In a further aspect of the invention there is provided a polyhipe scaffold as hereinbefore defined comprising multiple cells characterised by growth in three dimensions as hereinbefore defined in polymer zones as hereinbefore defined.

The scaffold including cells may comprise any of the properties as hereinbefore defined.

Multiple cells may be any desired cell type selected from human, animal and plant cells. Preferably cells are human or animal cells and are representative of multiple cell types present in any organ, system or part of the human or animal body. Cells are preferably selected from isotropic tissue and bone cells present in cartilage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells and the like. Cell type includes for example fibroblasts, chondrocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes, neurons, myoblasts, macrophages and microvascular endothelium cells.

In a further aspect of the invention there is provided a biologically active system comprising a polyhipe scaffold and multiple cells as hereinbefore defined adapted to provide normal cell functioning associated with a natural biologically active system present in the human or animal body. A biologically active system may comprise the polyhipe scaffold intact or in

partially degraded state as hereinbefore defined adapted for fusion or assimilation into an environment.

In a further aspect of the invention there is provided a method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth in known manner. The method of the invention may include any technique for growth promotion, positional control and the like adapted to provide a structural system as hereinbefore defined.

In a further aspect of the invention there is provided a polyhipe scaffold adapted for multiple cell growth as hereinbefore defined, comprising multiple cells as hereinbefore defined or a biologically active system as hereinbefore defined for use as an implant *in vivo* in the human or animal body or as modules for *in vitro* studies mimicking a part of the human or animal body or for use in a growth environment for further systems as hereinbefore defined, for example for the growth of organ cells in the cell side of the module in order to simulate organs.

In a further aspect of the invention there is provided an organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in PHP. The module is suited for growth of specific organ cells in the PHP and/or the channels. Some cells may be in contact with a specific microchannel and all cells will be capable of intercell communication.

According to this aspect of the invention *in vitro* organs may be produced using the micro capillary interconnects of the scaffold as hereinbefore defined for nutrient circulation and waste product removal while growing cells in the bulk pores and additionally or subsequently when anisotropic cell growth is needed for example to mimic cardiovascular systems and the like, employing circulation of nutrients and waste products removable through the bulk pores of the micro porous scaffold while growing cells in the micro capillary interconnects. It is a particular advantage of the invention that this allows growth and sustains cell functioning for prolonged periods. The use of the scaffolds and systems as hereinbefore defined as *in vitro* organs may be harnessed for testing of pharmaceuticals and any substances to be introduced into the human or animal body, for genetic engineering, for studying mechanisms associated with disease and the like under controlled conditions and the like. This is particularly advantageous in eliminating the need for animal testing. *In vitro* modules as hereinbefore defined for specific types of multiple cells and applications may be provided and may be associated with instrumentation to provide the necessary physiological conditions.

The module is suited for growth of specific organ cells in the PHP and/or the channels. Some cells may be in contact with a specific microchannel and all cells will be capable of intercell communication.

In order to mimic the cells natural environment within an organ, a number of facilities have to be provided for the physiologically correct cell function. These facilities can be summarised as:

1. blood or nutrient supply microcapillaries;
2. cell expression collection channels;
3. neural stimulation;
4. microcellular support for the co-culture of support cells;
5. biocompatibilty and controlled biodegradability; and
6. controlled mechanical, chemical and electrical characteristics of support.

It is possible to allocate a set of micro-capillary channels which are separated by cell support material (bulk of the organ support) to facilitate (1)-(3). In such an organ support system, cells are within a few hundred micron of these micro-capillaries. In each set of capillary network as well as in the bulk, mean pressure can be regulated so that the cell expression products are collected in the designated set of capillaries and subsequently recovered. Neurons can be grown in the designated set of micro-capillaries and electrically stimulated through an external micro-chip. Since the cell expression is affected by the external stresses (physical stresses such as cyclic strain and temperature, chemical stresses such as drug/toxin in blood/nutrient, electrical stimulation through the nerves) it is possible to obtain different cell expressions depending on the external stress which can be controlled very accurately in an *in vitro* organ. *In vivo*, the range and duration of external stresses are limited and there is always interference from the other cells. Therefore, *in vitro* organs with physiological facilities are useful to extend the scope of cell response to external stresses. In this respect, *in vitro* organs can be viewed as Biomedical Reactors which allows the synthesis of novel proteins and understanding of disease processes.

One set of capillaries can be used for seeding so that cells do not need to travel a long distance in order to occupy the available space. After seeding, these micro-capillaries can be used for other purposes.

It is possible to design an organ support system which allows the co-culture of support cells in close proximity to the main organ cells. PHP emulsions with differing pore and interconnect sizes can be co-extruded in a desired configuration (i.e. concentrically or side-by-side). As found in this invention, cells do not penetrate if the pore and interconnect sizes are too large or too small with respect to the cell size. Therefore, each type of cell will be able to choose the optimum pore structure for support and growth.

The electrical stimulation of growing nerve cells in a micro-capillary can be achieved by coating the micro-capillaries with conductive polymers. In the absence of any neural network for electrical stimulation, controlled electrical fields within the support system can be created by using a network of carbon fibres as conductors.

It should be appreciated that the invention derives from the finding that polyhipe scaffolds as hereinbefore defined may be provided with controlled pore dimensions and diameters in zones as hereinbefore defined, such that for example one cell type penetrates the scaffold and a second cell type may grow on the surface. This enables controlled cell growth and positioning in manner to create a multiple cell structure which is adapted to co-operate to provide biological activity.

In a further aspect of the invention there is provided a process for the preparation of microcellular polyhipe natural or synthetic polymers as hereinbefore defined comprising in a first stage the formation of a high internal phase emulsion (HIPE) of dispersed phase in continuous phase, wherein the dispersed phase may be void or may contain dissolved or dispersed materials, and monomers, oligomers and/or pre-polymers are present in the continuous phase, homogenisation and polymerisation thereof, by means of in the first stage introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature to achieve an emulsion, and subsequently homogenising for controlled period under controlled deformation and polymerising, under controlled temperature and pressure.

The process of the invention enables a vast range of pore sizes to be obtained by controlling processing technique and compositional conditions and in particular as hereinbefore defined to obtain Type 1-4 micro-pores. The pore sizes are obtained in roughly 3 groups: they are; small pore size: 1-10 μm; large pore size: 11-200 μm and very large pore size: 201-10,000 μm.

Very small pore size emulsions (approaching 0.5 μm) are obtained using very high deformation rate flows in which the flow is predominantly extensional and the emulsification temperature is as low as possible. Large pore size emulsions (approaching 200 μm) are obtained at high temperatures and just above the critical deformation rate below which the emulsion will fully or partially invert, for example to an oil-in water type system. The critical deformation rate may be determined by varying, for example the rate of addition or rate of deformation during mixing for a given system. These

emulsions should also be processed in a short time using predominantly shear flows.

Very large pore emulsions (approaching 10,000 μm) are obtained through the method of controlled pore coalescence during polymerisation. There are two methods of achieving controlled coalescence: 1) by adding into the aqueous (dispersed) phase a known amount of water soluble polymer or 2) by adding 'filler' solutes into the continuous oil phase. In both methods, the concentration and the type of these additives are important. If the concentrations are low, these additives result in polyhipe polymers in the range of 1-200 μm with some desired properties. If the concentration is above a certain value, the coalescence pores start to form. In this case, the pore size is dictated by the size of the pores before the start of coalescence, temperature of polymerisation and concentration, molecular weight and type of additive.

The emulsion may be obtained from any desired immiscible phases forming a continuous and a dispersed phase, preferably from aqueous and non-aqueous phases, more preferably aqueous and oil phases. The emulsion obtained may be an aqueous in oil emulsion or oil in aqueous emulsion.

The process according to the invention may be used for the preparation of any desired polymers as hereinbefore defined.

According to this aspect of the present invention, it has surprisingly been found that by means of controlled dosing the dispersed phase into the continuous phase, it is possible to achieve the desired emulsion. In a batch

mixer, dosing of the dispersed phase is preferentially conducted from the bottom of the mixer, using either single or multiple entry points. Multiple entry feed resulted in larger pore emulsions. If the dosing rate was very fast, mixing created by the emerging jet of aqueous phase was too severe and therefore the emulsion pore size decreases. Therefore, this combination of multiple-feed points with a relatively prolonged dosing created large pore emulsion. After the completion of dosing, the emulsion should be homogenised but if the homogenisation period was long, pore size decreased.

Controlled mixing as hereinbefore defined may be critical or extended. Critical mixing is sufficient mixing to cause the dispersion of aqueous phase into the oil phase without phase inversion. Critical mixing is obtained by use of a homogeneous mixing field whereby pore size is substantially uniform preventing emulsion breakdown and phase inversion.

Mixing may be by any means suited to provide a homogenous mixing field substantially throughout the total volume of the two phases and is preferably by multiple blade, multiple jet and the like mixing.

It has surprisingly been found therefore that, contrary to the teaching of US 5,071,747, the achievement of a stable emulsion with large pore diameter is obtained by minimising the intensity of mixing. According to the invention it has been found that by means of dosing, homogeneous mixing and the like a stable emulsion may be obtained dispensing with the need for intense mixing.

Dosing, emulsifying and homogenisation may be conducted at any suitable temperature depending on the pore size in the final polyhipe polymer. If the desired pore size is large, the preferred temperature is high, but nevertheless, below the boiling point of the lowest boiling phase. An aqueous phase as the lowest boiling phase, boils at around 100°C. It has been found that the process of the invention employing emulsification temperature of 60°C or greater results in polymers being obtained having pore size in excess of 60 micron. Increase of emulsification temperature above 60°C results in dramatic increase in pore size by an amount greater than that achieved for a similar increase in temperature below 60°C.

The maximum emulsification temperature may be greater than the normal boiling temperature of the lowest boiling phase, for example the lowest boiling phase may include any suitable component adapted to raise the boiling point. Preferably the aqueous phase includes an electrolyte which is stable at 100°C and is potentially inert. By this means the process is preferably carried out with use of homogenisation temperature in the range 60-150°C, more preferably 80-140°C, most preferably 80-120°C.

Emulsification and subsequent polymerisation can be carried out at temperatures above the normal boiling point of the aqueous or continuous phase materials, by increasing the pressure above atmospheric using closed continuous processing equipment.

The process may be carried out with use of additional aqueous or oil phase initiators, cross linking agents, fillers and the like and it is preferred that these are stable at the maximum operating temperature as hereinbefore

defined. Selection of initiators, cross linking agents and the like is made with reference to the acceptable viscosity of the phases for emulsifying and homogenising. It may be acceptable to reduce the amount of cross linking agent required by use of a proportion of pre polymers and partly cross linked pre polymers, optionally with the use of a suitable oil phase filler to increase oil phase volume and reduce effective viscosity.

Preferably the process of the invention is characterised by use of an initiator in the oil phase, together with or in place of an aqueous phase initiator as known in the art.

Preferably the process is carried out with use of oil phase fillers and allowing operation at high emulsification temperature and with use of minimum cross linking agent. This has the further advantage that oil phase filler such as high boiling point hydrocarbon may be leached out after polymerisation creating nano-pores and increasing the size of the interconnects.

Electrolytes may be for example calcium chloride or minerals such as hydroxyapatite.

Aqueous phase initiators may be employed for operation at lower temperatures and include sodium or potassium persulphate.

For operation at elevated temperature above 80°C oil phase initiators are preferably used for example, 1,1-azobis (cyclohexanecarbonitrile).

Cross linking agent may be for example divinylbenzene (DVB). If the polyhipe is required to be biodegradable, hydrolyzable crosslinks can be obtained. These crosslinking agents are ethylene diacrylate, N-N'-diallyl tartardiamide, N-N (1,2, dihydroxyethane) - bis -acrylamide, and N-N'-N" - triallyl citrictriamide. However, in this case of biodegradable crosslinkers, the polymer itself should be biodegradable. These polymers are poly (lactic acid), poly (glycolic acid), poly ε-caprolactam and polyacrylimide.

When water soluble polymers are needed to form the microcellular structure, they need to be crosslinked . In this case of such polymers, monomer (such as acrylamide) is dissolved in water and a HIPE emulsion is formed dosing this monomer solution into a hydrocarbon liquid such as hexane or toluene in the presence of suitable surfactant, initiator and crosslinker.

Proteins and cellulose can also form microcellular structures. In this case, these materials together with a suitable emulsifier are dissolved in a suitable aqueous phase (water for proteins and Schweitzer's reagent, Cu $(NH_3)_4$ $(OH)_2$ for cellulose) and dosed into a hydrocarbon liquid to form a water continuous HIPE. Crosslinking is achieved by immersing the HIPE into a solution of glutaraldehyde (for proteins) or acid solution (cellulose).

Oil phase filler may be any high boiling point hydrocarbon, another monomer, or macromonomer, reactive or inert polymer, solid particles, or their combinations.

The process may include introduction of any suitable modifier as hereinbefore defined prior to or subsequent to polymerisation. For example

minerals such as hydroxyapatite may be introduced in the dispersed aqueous phase and dosed into the continuous phase as hereinbefore defined. Alternatively a post polymerisation modification stage is employed, which may simply take the form of removing surfactant, electrolyte and unreacted monomer, coating, further polymerisation or reaction on the existing polymer surface. Modifying agents and modification techniques are shown in the art.

Polymerisation is carried out under known conditions of time and temperature for the respective monomer, oligomer and or prepolymer to be polymerised, as known in the art.

In a further aspect of the invention there is provided a microcellular polyhipe natural or synthetic polymer in the form of a homogeneous cross linked open cellular material having porosity in excess of 75% comprising pores and pore interconnects formed by polymerising a high internal phase emulsion (HIPE) as herein before defined wherein average pore diameter is in excess of 50 micron. Preferably average pore diameter is in excess of 100 micron, more preferably in excess of 150 micro and is selected to be suitable for the desired polymer application. Pore diameters up to 10,000 micron are envisaged. Interconnect diameters are as hereinbefore defined.

In a further aspect of the invention there is provided polyhipe polymer as hereinbefore defined having average pore size as hereinbefore defined in the range 1-10,000 micron, wherein the polymer is modified during polymerisation thereof or post polymerisation to be electrically conducting, degradable, or comprises mineral distributed throughout the matrix or as a surface coating.

In a further aspect of the invention there is provided an apparatus for the preparation of a polymer according to the invention comprising a mixing vessel adapted to contain the continuous phase having multiple inlets for the introduction by dosing of the dispersed phase as hereinbefore defined, and comprising means for homogeneous mixing as hereinbefore defined and comprising temperature elevating and regulating means.

In a further aspect of the invention there is provided a method for manufacturing an organ support module as hereinbefore defined. The method employs one or more stages as hereinbefore defined for creating Type 1-4 pores.

The invention is now illustrated in non limiting manner with reference to the following examples and figures wherein:

Figures 1a – 2b are schematics and a cross-section of organ support systems employing the invention.

Example A - preparation of polyhipe, using apparatus according to the invention.

The preparation of the emulsion was carried out in a batch mixer from an oil phase and an aqueous phase, dosed at a predetermined rate while the emulsion was stirred at constant rotational speed. Dosing rate, deformation rate, mixing rate were predetermined as a function of volume of respective

phases, diameter of the batch mixer and of the impellers, rotational speed of the impellers and homogenisation time.

In order to eliminate the differences in performance of different mixing conditions we characterise the mixing through:

Dosing rate $R_D = \frac{V_A}{t_D}$

Deformation rate during dosing $R_E = V_A / (t_D V_0)$

Mixing rate $R_M = D_1 \Omega / D_0$

Where:

V_A = Volume of aqueous phase added over a period of time t_D

V_0 = Volume of the oil phase placed in the batch mixer

D_1 = Diameter of the impellers

D_0 = Diameter of the batch mixer

Ω = Rotational speed

We also define t_H as the homogenisation time and t_T as the total mixing time.

$t_T = t_D + t_H$

After the preparation of the emulsions under a given set of conditions as described in each of the following examples, the emulsion was allowed to polymerise at 60°C for 8 hours. Samples were further modified as described in the following examples.

The pore and interconnect sizes were determined using scanning electron microscope, using thoroughly dried and washed samples.

Example A1 - the effect of operating condition on the pore and interconnect size.

The oil phase contained 78% styrene, 8% DVB monomer and cross-linking agent and 14% Span 80 surfactant sorbitant monooleate while the aqueous phase contained 1% potassium persulphate. Two flat paddle impellers (8 cm in diameter and 1.4 cm in width) were used in a mixing tank of 8.5 cm diameter. Impeller separation was 1 cm. 25 ml of oil phase is placed at the bottom of the tank and 225 ml of aqueous phase was dosed using 16 feed points. Temperature of the aqueous phase was ranged from -1.0 to 80°C.

Table 1 - The effect of operating conditions on the pore and interconnect size.

Temp	Pore	Interconnect	Dosing	Homogenisation	d/D

(degC)	Size(D) (μm)	Size(d) (μm)	time(s)	time(s)	
5	34	9	40	60	0.26
25	37	14	40	60	0.38
60	65	22	40	60	0.33
80	141	37	40	60	0.26
25	102	25	40	20	0.25
25	72	21	30	20	0.29
25	67	22	25	20	0.33
25	148	43	60	20	0.29

Example A2 – Effect of water soluble polymers on pore and interconnect size in controlled pore coalescence.

Emulsions were prepared using 78% styrene, 8% DVB and 14% Span 80 as the oil phase. The aqueous phase contained 1% potassium persulphate and varying amounts of water soluble polymers, sodium carboxy methyl cellulose (CMC) or polyethylene oxide (PEO). Emulsification is at 25°C, water phase volume is 85% and the aqueous phase is delivered through a single inlet. The processing conditions were: $t_D = t_H = 600$ seconds, $R_D = 0.70$ ml/s, $R_E = 0.0067\ s^{-1}$ and $R_M = 4.7\ s^{-1}$.

Table 2 - The effect of water soluble polymers on pore size in controlled pore coalescence

Relative Molecular Mass	Water Soluble Polymer Concentration wt% of aqueous phase	Pore Size (μm)
Control	0	18
Sodium carboxylmethyl cellulose (CMC)		
90,000	0.5	22
90,000	1.0	260
250,000	1.0	1200
Polyethylene oxide (PEO)		
200,000	1.0	420
400,000	1.0	4300

Example A 3 - In-situ surface coating with hydroxyapatite

Emulsions were prepared using: (A1) 78% styrene, 8% DVB and 14% Span 80 or (A2): 15% styrene, 60% 2-ethylhexyl acrylate, 10% DVB and 15% Span 8. The aqueous phase contained either; (B1): 1% potassium per sulphate or (B2): 1% potassium per sulphate 0.5% hydroxyapatile (HA) and 15% phosphoric acid which is used to dissolve the hydroxyapatite (HA). Emulsification is carried out at 25°C, with water phase volume at 85% and the aqueous phase is delivered through a single inlet. The processing conditions were $t_D = t_H = 600$ seconds, $R_D = 0.83$ ml/s, $R_M = 4.7s^{-1}$ and $R_E = 0.0067\ s^{-1}$. After the emulsification and polymerisation, samples containing hydroxyapatite were soaked in 1M NaOH to precipitate hydroxyapatite. After precipitation, the samples were cleaned in water to retain pH = 7 and IPA and examined for their structure.

Table 3 - The effect of hydroxyapatite on pore and interconnect size.

Description	Oil and Aqueous Phase Compositions			
	A_1 B_1 (Rigid PHP)	A_1: B_2 (Rigid/HA PHP)	A_2: B_1 (Elastic PHP)	A_2; B_2 (Elastic/HA PHP)
Pore size D (μm)	22	38	9	17
Interconnect size d (μm)	2	11	1.5	6
d/D	0.09	0.29	0.17	0.35

Samples with compositions (A_1; B_1) and (A_1; B_2) are examined under SEM and their surface elemental analysis indicated uniform distribution of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) as shown in Table 4.

Oil phase containing just styrene is referred to as rigid PHP and when oil phase contains 2-ethylhexyl acrylate, the resulting composition is referred to as elastic PHP.

Table 4 - Elemental analysis of the fracture surface of two polyhipe polymers with compositions (A_1; B_1) and (A_1; B_2)

Component	Unmodified PHP (A_1:B_1) - wt%	Hydroxyapatite coated PHP (A_1; B_2) wt%
Carbon	82	74
Oxygen	12	21.4
Calcium	-	3.2
Phosphorus	-	1.4

This in-situ hydroxyapatite coating of polyhipe is preferred since post-polymerisation soaking of the material in hydroxyapatite solution and subsequent precipitation appeared to form large but localised hydroxyapatite crystals within the polyhipe pores.

Example A 4 – Effect of oil phase composition on pore and interconnect size

An emulsion was prepared as described above, including 0.5% of oil phase initiator, 1,1'-azobis(cyclohexanecarbonitrile). Total volume and viscosity were substantially unchanged. The emulsion was processed and polymerised as described above but using conventional mixing times and rates as known in the art (Dosing rate R_D = 0.71ml/s, mixing rate, R_M = 4.7 s^{-1}, mixing time $t_D = t_H$ = 600s), and the material properties evaluated. The results are shown in Table 5.

Table 5

Sample	Pore Size,D (μm)	Hole Size, d (μm)	d/D
Control polyhipe	15.8	2.3	0.15
Polyhipe with initiator	22.7	4	0.18

Example A 5 - Effect of oil phase soluble initiator on hydroxyapatite modified polyhipe.

An emulsion was prepared using 0.5 wt% hydroxyapatite solution (pH = 2.5 adjusted with phosphoric acid) without any aqueous phase initiator. The oil phase contained 77.5% styrene, 8% DVB, 14% Span 80 and 0.5% azobisisobutyronitrile (AIBN) as oil phase initiator.

All other experimental conditions were the same as Example 3. In Table 6 the hydroxylapatite modified polyhipe is compared with the corresponding material in Example 3 (i.e., rigid/HA PHP).

Table 6

Sample	Pore Size D (μm)	Interconnect Size d (μm)	d/D Ratio
Rigid/HA	38	11	0.29
Rigid/HA AIBN initiated	30	12	0.40

The above table indicates that the presence of oil phase initiator does not substantially affect the pore and interconnect sizes. However, the elemental

analysis of the polyhipe surfaces using EDAX (energy dispersive analysis with X-rays) show that the presence of HA is more pronounced if oil phase initiator is used. These results are shown in Table 7.

Table 7

Component	Overall analysis on HA modified polyhipe with AIBN	Overall analysis on HA modified polyhipe
Carbon	63.09	74
Oxygen	22.49	21
Phosphorus	2.98	2
Calcium	9.70	3

The concentration of calcium ions is greater than for the control which did not contain AIBN

Example A6 – Effect of silica particles in aqueous and oil phases

Hydrophilic silica (Aerosil 380, particle size 7nm) was incorporated in the aqueous phase while hydrophobic silica (Aerosil R812, particle size 20nm) was incorporated in the oil phase. These silica samples were obtained from Degussa, Germany. In each case silica loading was 0.5, 1.0, 2.0 or 5.0 % by weight. Phase volume of the aqueous phase was 90% and the processing conditions were: Dosing rate R_D = 0.375 ml/s; mixing rate R_M =4.7 s^{-1}, mixing time t_D = 600 s, t_H =1200 s. The oil phase initially contained 15% styrene, 62% 2-ethylhexyl acrylate, 8% DVB, and 15% Span 80. Hydrophobic silica was added into this mixture at various levels. The aqueous phase contained 1% potassium persulphate. When hydrophilic silica was used in the aqueous phase, there was no silica in the oil phase and

the original oil phase composition was used as above. The results are shown in Tables 8 and 9.

Table 8. Effect of silica on the pore and interconnect size of polyhipe polymer when silica is present in the oil phase only.

Silica in oil phase (wt%)	0	0.5	1.0	2.0	5.0
Pore size, D (μm)	10.7	14.3	21.9	37.2	60.7
Interconnect size, d (μm)	4.0	3.2	2.9	3.2	3.43
d/D	0.37	0.22	0.13	0.09	0. 06

Table 9. Effect of silica on the pore and interconnect size of polygipe polymer when silica is present in the aqueous phase only.

Silica in aqueous phase (wt%)	0	0.5	1.0	2.0	5.0
Pore size, D (μm)	10.7	17.6	28.9	36.2	Coales-cence pore formation
Interconnect size, d (μm)	4.0	3.6	23.4	1.9	
d/D	0.37	0.21	0.12	0.05	

Example B 7 – Multiple cell growth in polyhipe

Chondrocytes (obtained from bovine metacarphalangeal joint) human osteoblasts cells (HOB) and 3T3 fibroblast rat cells were obtained. Two samples of polyhipe, without and with HA were cut into discs, washed and thoroughly sterilised. Thermanox discs were used as control. The discs were soaked in the respective cell culture medium for 24 hours and placed in well plates. 3T3, HOB and chondrocytes cells were each seeded onto the discs at a densities of 100,000, 100,000 and 500,000 cells per ml respectively. The

plates were then incubated at 37C in 5% CO2 for up to three weeks. Samples were examined after 1,7,14 and 21 days by SEM and for histology.

Results

The 3T3 which grew on the rigid polyhipe can be seen to have multiplied rapidly, by day 1 a confluent layer was present on both types of polyhipe. This layer remained throughout the duration of the experiment. From histology it could be seen that there was no penetration, in some samples by day 7 and in all samples by day 14 the cell layer had begun to leave the surface of the polyhipe.

The HOB cells showed well spread morphology on day 1 under SEM, the cells were bridging the interconnects on the surface of the polyhipe, opposed to growing into the interconnects. By day 7 all samples had a confluent layer on the surface. From histology was noted that there was slight penetration, only to the depth of one pore in the samples containing HA. The layer remained securely attached to the surface throughout the duration of the experiment.

The chondrocytes at time points of 1 and 7 days adhered to the polyhipe discs and showed rounded morphology on both types of polyhipe, compared to the Thermanox^{R} control where the cells had spread out and become fibroblastic in appearance. By day 14 there was signs of flatter cells and by day 21 there were confluent layers on some samples. The cells on the HA polyhipe could be observed to flatten at early time points compared to the unmodified polyhipe. From histology it was noted that from day 5 onwards

there was penetration in both polyhipes and cells were present within the 3D structure, however the HA polyhipe had greater penetration and visually seemed to contain more cells. The cells which had penetrated the unmodified polyhipe were fewer in number however were retaining their rounded morphology.

From the results of Safinin O staining it can be shown that healthy chondrocytes were present in both the centre and the periphery of the polymer matrix. There were viable cells secreting GAG both in the polymer and on the periphery.

The rate of chondrocyte growth studies (as obtained from DNA assays) indicate that the hydroxylapatite modified polyhipes yield significantly faster growth compared with unmodified polyhipes as shown in Table 10.

Table 10

Type of Assay	Cell Lysis (mg DNA/ml)	
Time (day)	PHP	HA-PHP
1	3.1	3.2
5	3.6	4.4
10	3.6	9.5

Example B8 – Effect of Pore Size on Cell Growth

Six types of styrene / 2-ethylhexyl acrylate Polyhipe Polymers (containing hydroxapatite coating) were produced with average pore size of 8, 17, 24, 31, 34 and 89 μm. The interconnect size in all cases were similar. These

samples were then seeded with chondrocytes and the effect of pore size was noted. The results are shown in Table 11.

Table 11. Effect of PHP pore size on the cell penetration and collagen II production after 21 days of cell culture.

Pore size, D (μm)	8	17	24	31	45	89
Interconnect size, d (μm)	3	5	6	6	7	7
Depth of Penetration (μm)	17	508	570	367	67	45
Relative Collagen II Production	23	48	52	42	12	9

The production of Type II collagen is an indication of the correct physiological cell function. This example indicates that, for chondrocytes which have cell size of approximately 10 μm, the optimum pore size is approximately 25 μm. If the pore size is too low, the cells can not penetrate the polymer and if the pore size is too large, the cell morphology changes from rounded to a flat and fibroblastic appearance. These fibroblastic cells proliferate rapidly and form a layer on the surface rather than penetrating the polymer.

Another measure of extra-cellular matrix production is the yield of glycosaminoglycan (GAG). The concentration of GAG was determined by a colorimetric assay. The effect of pore size in the production of GAG was evaluated using the cell culture studies summarised in Table 10. The GAG

production studies were also compared with the Tissue Culture Plastic (TCP). The results are shown in Table 12.

Table 12. Effect of pore size on the production of GAG after 21 days of cell culture. Results are also compared with the cell culture studies using tissue culture plastic (TCP).

Pore size, D (μm)	8	17	24	31	45	89	TCP
Interconnect size, d (μm)	3	5	6	6	7	7	0
Relative GAG Production	192	720	784	528	384	400	352

Example B 9 – Macrophage Activity on Sulphonated Polyhipe Polymer

The biocompatibility of the materials can be tested by exposing them to macrophages. The response of macrophages to a material is assessed by a) Morphological changes to macrophages, b) Hydrogen peroxide production by macrophages and c) Beta glucuronidase production by macrophages. Macrophages are spherical *in vitro* and any deviation from this response is a negative response. Excessive production of hydrogen peroxide and beta glucuronidase are also negative responses.

A styrene / 2-ethylhexyl acrylate Polyhipe Polymer is produced. Some of this material is sulphonated (degree of sulphonation is 12%) and subsequently neutralised using sodium hydroxide. Macrophages are seeded

on these polymers and their morphological and hydrogen peroxide and beta glucuronidase production capacities are assessed after six hours. The results are shown in Table 13.

Table 13. Effect of substrate on macrophage activity

Substrate Type	Morphology	Relative Hydrogen Peroxide Production	Relative Beta Glucuronidase production
PHP	Round	370	47
Sulphonated PHP	Flat	975	684
TCP*	Round	295	93

- **TCP = Tissue Culture Plastic**

Table 13 indicates that sulphonated PHP yields very high level of negative activity and therefore such polymers may be used as negative standard in which the activity is reduced as the degree of sulphonation is decreased.

Example C - Fabrication of an Organ Support System

Figures 1a - 2b illustrate micro- and macroscale capillary and channel configurations in polyhipe modules obtained by the method of Example C.

In a cell support system, we can assume that only Type-1 pores (basic pores) are present with probably a set of micro-capillaries which provide an effective cell seeding and subsequently act as nutrient channels. In this case, these micro-capillary channels need not to be closely packed and they can have large diameters approaching 500 micron. Such cell support systems can have the shell-and-tube type of arrangement and can be constructed to have a cylindrical or square or rectangular cross-section. This arrangement is similar to the hollow fibre bundles except that the shell side constitutes the bulk PHP and micro-capillary/bulk PHP interface is very thin (0.5-5micron).

In an organ support system there is more than one type of micro-capillary is present. In a cubic or polyhedric organ support, cartridge, the opposing faces can be connected to each other with micro-capillaries. For the simplest case, a cubic or cuboidal organ support cartridge with three types of micro-channels are illustrated in Figures 1a, b. Figure 1a, 3D representation of the micro-capillaries are shown (only 3 types of capillaries are illustrated) while in Figure 1b, cross-section is illustrated showing all the capillaries and PHP in the bulk. Here, the channels are denoted as A-channels, B-channels, and C-channels.

The following procedure is used to obtain a 3D-network of fibres in a mould which is subsequently filled with the emulsion of the PHP and polymerised afterwards. However this procedure is not unique but it is intended to illustrate the fabrication technique of the in vitro organ support systems.

1. Strands of desired diameter metallic rods or fibres are sandwiched between two sheets of polymer using either a hot press or two roll-

mills (calendars). The metallic fibres or rods are parallel to each other and they will eventually create the A-channels. These sandwiched panels are cut to a desired size and optionally stamped with a die to form holes which will eventually house the metal rods leading to the formation of the B-channels. Several of these sandwiched panels are produced in this way.

2. The above procedure is repeated using desired diameter metallic rods or fibres in order to obtain the C-channels. If the diameters of the A- and C-channels were to be the same, there is no need to have separate fibres/rod sandwiched panels.

3. These panels are laid in a mould on top of each other with two types of thin metallic spacers between them. The first type of spacer secures the fibers in place while the second type can be removed when the fiber assembly is compressed in the direction of the B-channels. The orientation of the fibres which will form the A- and C-channels are perpendicular to each other. The mould already contains metallic rods which will form the B-channels. The thickness of the thin metallic spacers determines the separation of the A- and C-channels.

4. These panels are securely tightened and placed in an oven which allows the tightening of the fibre/rod layers as the temperature of the oven is raised gradually above the melting point of the polymer used in the sandwiched panels. The temperature is further raised in order to burn off the polymer completely leaving a 3D network of fibres/rods.

5. These fibres/rods may require surface coating with polymer in order to create a desired capillary/bulk interface. For example, these template fibres/rods can be coated with hydrophobic polymers to obtain closed pore structure at the interface when styrene based PHP polymers are produced. If the template fibres/rods are coated with water soluble polymers such as polyethylene oxide, Type-2 pores may be obtained at the interface. When metals are not coated, open pore structure is obtained at the interface.

6. The mould is filled with the emulsion slowly under vibration in order to obtain complete filling. The mould is further compressed in the direction of the B-channels by removing the type-2 metallic spacers, thus allowing the escape of the excess emulsion. Emulsion viscosity should be as low as possible and the emulsion should be re-circulated during filling.

7. After mould filling and polymerisation, the template rods are removed and the fibres are dissolved in acid thus exposing the microcapillary channels.

After the fabrication of the organ support cartridge, it is washed in water and neutralised and subsequently washed with ethanol to remove any residual monomer and re-washed with water and dried before being sterilised ready for use as an *in vitro* organ support.

In the cuboidal modules of Figures 1a - 2b, there are three types of channels A, B, C. These are shown in Figures 2a and 2b, connecting the faces of a cube, indicated in Figure 2a as A-A', B-B' and C-C', respectively. A-channels may be carrying blood (or plasma, or nutrients) B-channels may be used to transport the cell products (cell expression, metabolic products) while the C-channels can be used to grow anisotopic cells such as nerve or muscle cells. Using a cuboidal module with eight faces and hence four types of micro-channels, one can also grow muscle cells together with nerve cells, still providing for blood and cell product collection. The space between the micro-channels will be occupied with a specific organ cell or connective tissue or tissue or stimulant.

Each channel can be made of different diameter and different packing density and they can be independently surface modified (for example made electrically conductive, or have different interface porosity or pore size, or different chemical structure). Instead of growing nerve cells, it might be sufficient to have for example, carbon fibres present in these channels to provide electrical conductivity.

CLAIMS

1. Micro cellular polyhipe polymer scaffold suitable for growth of living matter for biomedical applications,

comprising a homogeneous cross linked open cellular material defined by a bulk polymer matrix having a surface and an interface with an internal phase, and having porosity greater than 75% comprising emulsion derived pores of diameter in the range of 0.1 to 10,000 micron and emulsion derived pore interconnects of diameter in the range of up to 100 micron, wherein the scaffold comprises a plurality of discrete and/or interpenetrating zones:

at the polymer surface;

within its bulk matrix;

at the interface between polymer and internal phase; and/or

between adjacent but distinct pores and/or interconnects,

characterised by form and dimension of pore and interconnect type within each zone, and location of zones wherein adjacent or interpenetrating zones are distinguished by boundaries which may be between or contained within adjacent pores and/or interconnects in respective zones, whereby zones are suitable for regulating positioning and/or morphology of living matter

comprising controlled pore sizes in the range up to 0.5 μm, up to 300 μm, up to 10,000 μm, and/or up to nm size and comprising pore interconnects in the range up to 100 micron, and/or approaching 500 micron

with the proviso that in the case that pore type is present having size in the range 1 – 50 micron, at least one additional pore type is present in a range as herein defined other than 1 – 50 micron.

2. Microcellular polyhipe polymer scaffold according to Claim 1 obtainable by polymerising a high internal phase emulsion of an immiscible dispersed

phase in a continuous phase, wherein the dispersed phase is void or contains dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature and rate to achieve an emulsion of controlled pore size, and subsequently homogenising for controlled period under controlled deformation and polymerising under controlled temperature and pressure,

characterised in that controlled pore size up to 0.5 μm is obtained using very high deformation rate flows in which the flow is predominantly extensional and low emulsification temperature, pore size up to 300 μm is obtained using rate just above the critical deformation rate at which phase inversion takes place and high emulsification temperature, very large pore size up to 10,000 μm is obtained through the method of controlled pore coalescence during polymerisation, and nano-pore size up to nm is obtained through solvent extraction after polymerisation; and in that controlled interconnect diameter up to 500 micron is obtained by polymerising about a 3D network of fibres.

3. Microcellular polyhipe polymer scaffold according to Claim 1 *or 2* suitable for growth of living matter selected from cells, micro-organisms such as bacteria and virus and mixtures thereof.

4. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 3 comprising micro channels formed of pores with interconnects suitable for providing communication and penetration of living matter for anisotropic (directional) growth thereof.

5. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 4 wherein the walls of the micro-channels are (bio)degradable suitable for fusion of living matter in the (bio)degraded scaffold.

6. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 5 comprising in individual zones, pore and interconnect sizes in different ranges, suitable for co-culturing two or more types of living matter.

7. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 6 wherein ratio of interconnect to pore diameter is in the range $0 < d/D < 0.5$, preferably in the range $0.1 < d/D < 0.5$ when the pore diameter is approximately less than 200 micron

8. Microcellular polyhipe polymer scaffold as claimed in Claim 7 comprising extensive networks of elongate micro capillaries obtainable by moulding about fibrous inserts of diameter as low as 10 micron, throughout the scaffold or zones thereof, separated by the microcellular polymer wherein microcapillaries are suitable for blood or nutrient supply channels, expression channels for living matter and seeding of living matter.

9. Microcellular polyhipe polymer scaffold as claimed in any of Claims 7 and 8 wherein the interface between a microcapillary wall and the bulk polymer provides a thin surface layer of the order of 0.5-5 micron, forming a zone particularly suited for directional (anisotropic) growth of living matter.

10. Microcellular polyhipe polymer scaffold as claimed in Claim 9 as dependent on Claim 3 wherein the interface has smaller pore size than the bulk polymer wherein the zone is suitable for growth of cells forming a lining, for example cells lining the blood vessels or for growing endothelial cells on the

interface surface.

11. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 10 comprising a module of shell and tube type or cubic/polyhedric type with respect to direction and/or configuration of channels and/or microcapillaries.

12. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 11 comprising a surface coating, using coating materials introduced *in situ* during polymerisation or post polymerisation

13. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 12 wherein polymer is selected from proteins and cellulose, polyacrylamide, polyvinyl in rigid or flexible form, poly(lactic acid), poly(glycolic acid), polycaprolactone, poly (lactide/glycolide) and polyacrylimide.

14. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 13 wherein polymer comprises resiliently deformable or elastic material or is rendered resiliently deformable or elastic and is suitable for repeated stress and relaxation by means of oscillatory straining of the scaffold during cell growth facilitating rate of cell growth.

15. Microcellular polyhipe polymer scaffold as claimed in any of Claims 1 to 14 wherein polyhipe scaffold is electrically conductive or is rendered electrically conductive whereby it is suitable for conducting an electric current during cell growth, facilitating distinguishing certain cell types and promoting growth and fusion of particular cell types

16. A process for the preparation of a microcellular polyhipe polymer

scaffold as hereinbefore defined comprising in a first stage the formation of a high internal phase emulsion (HIPE) of an immiscible dispersed phase in a continuous phase, wherein the dispersed phase is void or contains dissolved or dispersed materials, and (co)monomers, oligomers and/or pre-polymers are present in the continuous phase, by means of introducing the dispersed phase by controlled dosing into the continuous phase with controlled mixing at controlled temperature and rate to achieve an emulsion of controlled pore size, and subsequently homogenising for controlled period under controlled deformation and polymerising under controlled temperature and pressure.

17. Process as claim in Claim 16 wherein controlled pore size of emulsions up to 0.5 μm are obtained using very high deformation rate flows, pore size up to 300 μm are obtained using rate just above the critical deformation rate at which phase inversion takes place, very large pore size up to 10,000 μm are obtained through the method of controlled pore coalescence during polymerisation, and nano-pore size up to nm are obtained through solvent extraction after polymerisation.

18. Process as claimed in Claims 16 or 17 comprising co-extrusion of polyhipe emulsions of differing pore and interconnect sizes eg concentrically or side-by-side.

19. Process as claimed in Claims 16 to 18 wherein the emulsion comprises aqueous and non-aqueous phases, preferably aqueous and oil.

20. A biologically active system comprising a polyhipe scaffold as defined in any of Claims 1 to 15 and living matter providing normal cell functioning

associated with a natural biologically active system present in the human or animal body, wherein living matter is selected from microorganisms or multiple cells selected from human, animal and plant cells, preferably selected from isotropic tissue and bone cells present in cartillage, cornea, marrow and the like, anisotropic cells such as nerve, muscle, blood vessel cells, of cell type selected from fibroblasts, chrondocytes, osteoblasts, bone marrow cells, hepatocytes, cardiomycytes neurons, myoblasts, macrophages and microvascular endothelium cells.

21. Method for growth of multiple cells in a polyhipe scaffold as hereinbefore defined in any of Claims 1 to 15 comprising providing cells on or in the scaffold in a controlled environment and providing a suitable nutrient adapted for growth and providing conditions for growth promotion and positional control.

22. Use of a biologically active system as defined in Claim 20 as an implant or in association *in vivo* in or with the human or animal body or as a module for *in vitro* studies mimicking a part of the human or animal body or for use in a growth environment, for example for the growth of organ cells in the cell side of the module in order to stimulate organs.

23. An organ support module comprising a cubic or polyhedric module of closely interwoven but not interconnecting channels immersed in a polyhipe scaffold as defined in any of Claims 3 to 15 suited for growth of specific organ cells in the polyhipe and/or the channels, wherein cells are optionally in contact with a specific microchannel and all cells are capable of intercell communication.

24. A method for manufacturing an organ support module as defined in Claim 23 comprising providing a polyhipe emulsion, providing a mould including inserts such as rods or fine fibres, pumping polyhipe emulsion and optional filler into the mould about the inserts and polymerising, with subsequent removal of inserts and optional filler to provide pores and interconnects, microcapillaries and nano pores in desired configuration.

25. The use of a polyhipe scaffold, a biologically active system, or organ support module as defined in any of Claims 1 to 15, 20 and 23 for the manufacture of contact lenses, dental fillings, cochlea implants, vascular supports including heart valves and cardiac pace makers and drug delivery skin patches.

ABSTRACT

Microcellular polyhipe polymer scaffold suitable for growth of living matter for biomedical applications, obtainable by polymerising a high internal phase emulsion, comprising a homogeneous cross linked open cellular material defined by a bulk polymer matrix having a surface and an interface with an internal phase, and having porosity greater than 75% comprising emulsion derived pores of diameter in the range of 0.1 to 10,000 micron and emulsion derived pore interconnects of diameter in the range of up to 100 micron, wherein the scaffold comprises a plurality of discrete and/or interpenetrating zones:

at the polymer surface;

within its bulk matrix;

at the interface between polymer and internal phase; and/or

between adjacent but distinct pores and/or interconnects,

characterised by form and dimension of pore and interconnect type within each zone, and location of zones wherein adjacent or interpenetrating zones are distinguished by boundaries which may be between or contained within adjacent pores and/or interconnects in respective zones, whereby zones are suitable for regulating positioning and/or morphology of living matter;

a biologically active system, or organ support module comprising the scaffold and methods and processes for preparation thereof and use thereof.

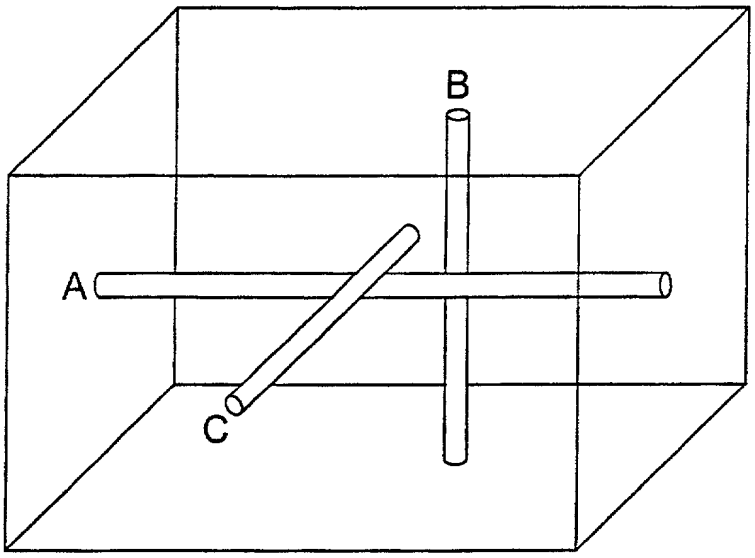


Fig. 1a

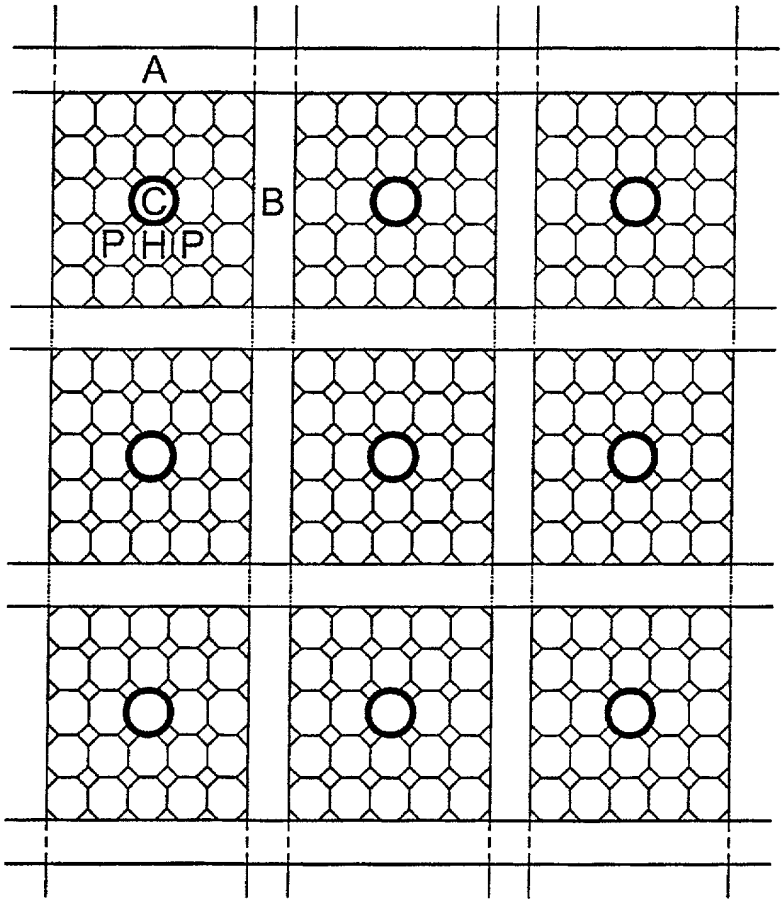


Fig. 1b

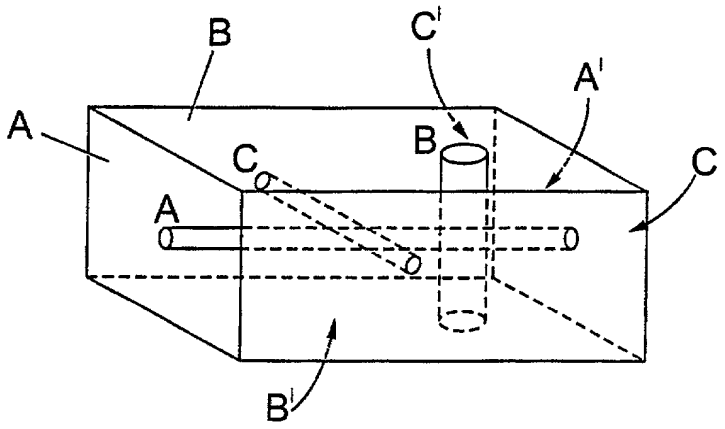


Fig. 2a

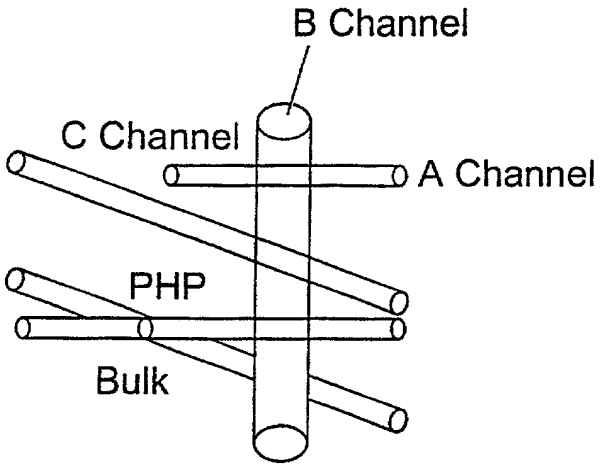


Fig. 2b

DECLARATION AND POWER OF ATTORNEY U.S.A.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION, NON PRIORITY; OR PROVISIONAL APPLICATIONS

FOR ATTORNEYS' USE ONLY
ATTORNEYS' DOCKET NO
P66710US0

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled

101 102

MICROCELLULAR POLYMERS AS CELL GROWTH MEDIA AND NOVEL POLYMERS

which is described and claimed in, [X] PCT International Application No **PCT/GB99/04076** filed **6 December 1999**

[ ] the attached specification [ ] the specification in application Serial No _____ filed _____

(if applicable) and amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

103

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
9826701.6	United Kingdom (GB)	5 December 1998	[X] Yes [ ] No
			[ ] Yes [ ] No
			[ ] Yes [ ] No

104

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

10 POWER OF ATTORNEY. As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); JOHN CLARKE HOLMAN (22,769), MARVIN R STERN (25,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L SCHERER (28,851); IRWIN M. AISENBERG (19,007); WILLIAM E PLAYER (31,409), YOON S. HAM (45,307) and NATHANIEL A HUMPHRIES (22,772)

SEND CORRESPONDENCE TO (CUSTOMER NO. 00136)	DIRECT TELEPHONE CALLS TO: (please use Attorney's Docket No.) (202) 638-6666
JACOBSON HOLMAN PROFESSIONAL LIMITED LIABILITY COMPANY 400 SEVENTH STREET, N.W. WASHINGTON, D.C. 20004	**JACOBSON HOLMAN** PROFESSIONAL LIMITED LIABILITY COMPANY

*Inventor(s) name must include at least one unabbreviated first or middle name

201 FULL NAME * OF INVENTOR	FAMILY NAME: AKAY	GIVEN NAME: Galip	MIDDLE NAME:	
RESIDENCE & CITIZENSHIP	CITY: SIDMOUTH GBX	STATE OR FOREIGN COUNTRY: UNITED KINGDOM	COUNTRY OF CITIZENSHIP: United Kingdom	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Rockshaw, Moorcourt close	CITY: Sidmouth, Devon	STATE OR COUNTRY: UK	ZIP CODE: EX10 8SU
202 FULL NAME * OF INVENTOR	FAMILY NAME: DOWNES	GIVEN NAME: Sandra	MIDDLE NAME:	
RESIDENCE & CITIZENSHIP	CITY: YORK GBX	STATE OR FOREIGN COUNTRY: UK	COUNTRY OF CITIZENSHIP: United Kingdom	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 16 ORCHARD PARK	CITY: MELBOURNE	STATE OR COUNTRY: YORK	ZIP CODE: YO42 4SL
203 FULL NAME * OF INVENTOR	FAMILY NAME: PRICE	GIVEN NAME: Victoria	MIDDLE NAME: Jane	
RESIDENCE & CITIZENSHIP	CITY: MELTON MOWBRAY GBX	STATE OR FOREIGN COUNTRY: UNITED KINGDOM	COUNTRY OF CITIZENSHIP: United Kingdom	
POST OFFICE ADDRESS	POST OFFICE ADDRESS: BERRY BOUGH COTTAGE 3 SANDY LANE	CITY: SCALFORD, MELTON MOWBRAY	STATE OR COUNTRY: LEICESTERSHIRE	ZIP CODE: LE14 4DS

1-02 2-02 3-02

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
Galip Akay	S. Downes	
DATE 4/6/2001	DATE 5/6/2001	DATE 25/7/01

[ ] Additional inventors are named on separately numbered sheets attached hereto.